

Shearwater Polymers, Inc. Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 10, at the end of the application.

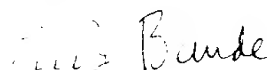
REMARKS

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-14, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Specification:**

Paragraph beginning at line 13 of page 5 has been amended as follows:

Figure 1 is a schematic of an approach for the disruption of the mouse CAR $\beta$  gene. The mouse cDNA is shown schematically, with the ATG denoting the start codon. The small bar immediately below the mouse cDNA represents the nucleic acid fragment that was used to probe Northern blots to ascertain whether the CAR allele has been disrupted. The DNA binding domain of CAR is indicated as forward hatched areas. The genomic structure of the CAR knockout construct is depicted below the cDNA schematic, shown with the Neo<sup>R</sup> gene inserted as used to construct the knockout mouse. The Neo<sup>R</sup> gene is transcribed in the direction of the arrow and is depicted as a reverse hatched area. The numbers refer to nucleotide positions in the CAR $\beta$  cDNA. The lower case nucleotides indicate the boundaries of the deleted region of the CAR $\beta$  gene (SEQ ID NOS:12 and 13).

Paragraph beginning at line 4 of page 6 has been amended as follows:

Figure 3 is shows the ability of 5 $\beta$ -pregnan-3,20-dione to increase the fluorescence polarization between rhodamine labeled ILRKLLQE, rhodamine-ILRKLLQE (SEQ ID NO:7), and a GST-CAR $\alpha$  ligand binding domain fusion protein. The concentration of 5 $\beta$ -pregnan-3,20-dione is on the x-axis.

Paragraph beginning at line 8 of page 6 has been amended as follows:

Figure 4 demonstrates that the androstane compounds 5 $\alpha$ -androst-16-en-3 $\alpha$ -ol and 5 $\alpha$ -androstan-3 $\alpha$ -ol are able to decrease the amount of fluorescence polarization seen with 5 $\beta$ -pregnan-3,20-dione, rhodamine-ILRKLLQE (SEQ ID NO:7), and a GST-CAR $\alpha$  ligand binding domain fusion protein (GST-CAR $\alpha$ ). The assay was performed with 5 $\alpha$ -androst-16-en-3 $\alpha$ -ol (filled squares) or 5 $\alpha$ -androstan-3 $\alpha$ -ol (filled circles) incubated in the presence of rhodamine-ILRKLLQE (SEQ ID

NO:7), and GST-CAR $\alpha$ . The IC<sub>50</sub> is the concentration at which 50% of the fluorescence polarization seen in the absence of the androstanes is inhibited. The assay was also performed with 5 $\alpha$ -androst-16-en-3 $\alpha$ -ol (open squares) or 5 $\alpha$ -androstan-3 $\alpha$ -ol (open circles) incubated in the presence of 5  $\mu$ M 5 $\beta$ -pregnan-3,20-dione, rhodamine-ILRKLLQE (SEQ ID NO:7), and GST-CAR $\alpha$ . The IC<sub>50</sub> is the concentration at which 50% of the fluorescence polarization seen with 5  $\mu$ M 5 $\beta$ -pregnan-3,20-dione in the absence of the androstans is inhibited.

Paragraph beginning at line 3 of page 8 has been amended as follows:

The term "CAR" refers to a member of the nuclear hormone superfamily known as the constitutive androstane receptor, the constitutively active receptor, CAR $\alpha$ , CAR $\beta$ , MB67, or the hamster CAR (SEQ ID NO: 8). Generally, a CAR will have encompasses amino acid sequences that are at least 75% identical to the predicted amino acid sequence of human CAR $\alpha$  (SEQ ID NO:1) (GenBank Accession No. Z30425), the predicted amino acid sequence of mouse CAR $\beta$  (SEQ ID NO:2) (GenBank Accession number AAC53349) or the predicted amino acid sequence of the hamster CAR gene (SEQ ID NO: 9).

Paragraph beginning at line 23 of page 27 has been amended as follows:

To form a chimeric receptor of the invention, the ligand binding domain and the DNA binding domain are linked together. Suitable methods of forming such linkages are known to those of skill in the art. For a review of methods for constructing fusion proteins between receptor ligand binding domains and DNA binding domains, *see, e.g., Mattioni et al. (1994) Methods in Cell Biology* 43(Pt A): 335-352. The linkage can be done using either recombinant or chemical methods. For example, a cysteine residue can be placed at either end of a domain so that the domain can be linked to another domain by, for example, a sulfide linkage. More typically, the ligand binding domains and DNA binding domains are joined by linkers, which are typically polypeptide sequences, such as poly-glycine sequences of between about 5 and 200 amino acids, with between about 10-100 amino acids being typical. In some embodiments, proline residues are incorporated into the linker to prevent the formation of significant secondary structural elements by the linker. Preferred linkers are often flexible amino acid subsequences which are synthesized as part of a

recombinant fusion protein. In one embodiment, the flexible linker is an amino acid subsequence comprising a proline such as Gly(x)-Pro-Gly(x) (SEQ ID NO:14) where x is a number between about 3 and about 100. A linker can also be a single peptide bond, or one or more amino acid residues. In other embodiments, a chemical linker is used to connect synthetically or recombinantly produced ligand binding domain and DNA binding domain subsequences. Such flexible linkers are known to persons of skill in the art. For example, poly(ethylene glycol) linkers are available from Shearwater Polymers, Inc. Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.